

The key role of humidity for the survival of cave beetles in a context of climate change*

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Abstract

Climate change is altering the environmental conditions of subterranean ecosystems, leading to warmer and drier caves across many regions. Despite growing evidence of the high heat sensitivity of cave-dwelling arthropods, the role of humidity as a limiting factor remains largely unexplored. To address this knowledge gap, we experimentally assessed the combined effects of temperature and humidity on the survival of four Pyrenean cave beetles (*Euryspeonomus eloseguii*, *Troglocharinus impellitieri*, *T. hustachei*, and *Stygiophyes ribagorzanus*). Adult beetles were exposed to eight combinations of temperature (11, 20, 23, and 25 °C) and relative humidity (100% and 75% RH) for seven days under controlled laboratory conditions. Survival was monitored daily, and Cox proportional-hazards models were used to analyze treatment effects. Our results showed a significant interaction between temperature and humidity across all species. By the end of the experiment, no individuals survived under 75% relative humidity at any temperature, nor at 23 °C or 25 °C in any humidity condition. Particularly, *E. eloseguii* and *T. impellitieri* were highly sensitive to humidity reduction, with no survival beyond 48 hours at 75% RH regardless of temperature. *E. eloseguii* also exhibited the lowest heat tolerance, with complete mortality at 20 °C even under saturated humidity. Our findings highlight the extreme vulnerability of subterranean beetles to desiccation and heat. Even moderate humidity reductions can drastically reduce survival, underscoring the need to consider combined climatic stressors when assessing the conservation status of subterranean species under climate change.

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Keywords

Climate change, ecophysiology, drought, hydric balance, Leiodidae, subterranean beetles

Introduction

In recent decades, climate change has driven global temperatures approximately 1.5 °C above preindustrial levels, with the past years ranking among the warmest on record (Rayner et al. 2003; Ripple et al. 2020; Calvin et al. 2023; Goessling et al. 2025). Alongside this warming trend, droughts are becoming increasingly severe and frequent, especially in Mediterranean regions, where biodiversity is already under significant stress due to rising temperatures and water scarcity (Vicente-Serrano et al. 2014; Noguera et al. 2020; Hermann et al. 2024).

While the ecological impacts of climate change are well documented in terrestrial, marine, and alpine ecosystems (Calvo et al. 2011; Chen et al. 2011; Grimm et al. 2013; Urban 2015; Ainsworth et al. 2016; Guerrero-Meseguer et al. 2017; Giménez-Benavides et al. 2018), subterranean ecosystems remain comparatively largely understudied. However, recent evidence suggests that these environments are also experiencing climatic shifts, generally becoming warmer and drier (Mammola et al. 2019a, b, 2022). Such changes may pose serious threats to cave-adapted species that have evolved under highly stable humid conditions. Subterranean invertebrates often have thin, highly permeable cuticles (Vittori et al. 2017), which along with their extremely low dispersal ability, makes them especially vulnerable to environmental change.

Previous research on physiological limits of subterranean species has focused primarily on the thermal tolerance of cave-dwelling arthropods, revealing narrow thermal niches and low heat tolerance across multiple lineages, such as cave beetles (Rizzo et al. 2015; Pallarés et al. 2019, 2021; Colado et al. 2022a), pseudoscorpions (Colado et al. 2022b), spiders (Mammola et al. 2018) or springtails (Raschmanová et al. 2018; Manee et al. 2025). In contrast, the role of humidity, a potentially critical factor for their survival, has received far less attention. Only a handful of early studies examined desiccation resistance and cuticular permeability in subterranean taxa, including the troglobitic spider *Lycosa howarthi* Gertsch, 1973 from lava tube caves in Hawaii (Hadley et al. 1981), the cave-dwelling cockroach *Blaberus craniifer* (Burmeister, 1838) (Herreid 1969) or the troglobitic springtail *Tomocerus problematicus* P. Cassagnau, 1964 (Vannier 1977). In such studies, troglobitic species showed higher water loss rates than their surface relatives. After these key early findings, the vulnerability of subterranean species to desiccation has remained largely unaddressed.

In surface insects, resistance to desiccation and heat is often mediated by the presence of waterproofing compounds in the cuticle (Gibbs 2002; Blomquist and Bagnères 2010). In contrast, the consistently humid conditions of subterranean habitats may have relaxed the selective pressure for such traits, potentially increasing the susceptibility of cave-adapted species to desiccation and elevated temperatures (Vittori et al. 2017; Riddell et al. 2023). Therefore, addressing the combined effects of temperature

and humidity is critical for understanding the vulnerability of subterranean organisms under climate change. To address this knowledge gap, the present study investigates the impact of temperature increase and reduced relative humidity on the survival of Pyrenean cave beetles of the tribe Leptodirini.

Methods

Target species and study area

The study species belong to the Pyrenean clade of the Western Mediterranean lineage of the tribe Leptodirini (Coleoptera, Leiodidae, Chovelinae), distributed in different areas of the Pyrenees and Cantabrian Mountains, from Navarra to Catalonia (Fresneda et al. 2024). We selected four species of this clade (Table 1) in accordance with the following criteria: i) abundance in each of their localities, ii) species of which thermal tolerance and phylogenetic data are available from previous studies (Rizzo et al. 2015; Sánchez-Fernández et al. 2016, 2018; Pallarés et al. 2021; Colado et al. 2022a; Fresneda et al. 2024), and iii) ease of access to the caves.

Adult specimens were collected between March and July 2023. In each one of the caves, a Bluetooth equipped Data-Logger (HOBO MX2301, Onset Computer Corporation, Bourne, USA) was used to record temperature and relative humidity. These were located at the areas with the highest density of individuals, and kept recording for 2 to 3 hours, while the specimens were captured.

Specimens were captured using mouth aspirators and immediately transferred into recipients with moss to maintain a high humidity. The recipients were transported to the laboratory in an electric portable refrigerator at the same temperature of the caves, which was continuously monitored.

Experimental conditions

Upon arrival at the laboratory in the University of Murcia, specimens were kept under conditions like those of their localities (11°C and 100% RH) for 72 hours, for initial acclimation. After that, 10 specimens per combined treatment of temperature and humidity (see below) were transferred to the experimental units consisted of plastic trays with the bottom covered in a 3 cm layer of plaster of Paris. These were placed in climatic chambers with temperature and humidity control.

Temperature treatments encompass a thermal range from current temperatures in the collection caves (11°C), to temperatures that have been shown to be sublethal (20°C) or lethal (23°C, 25 °C) within a 7 days exposure period at 100% RH in beetle species of this lineage within the same study area, according to previous studies (Rizzo et al. 2015; Pallarés et al. 2020; Colado et al. 2022a). Such studies used the same methodology employed here to estimate upper thermal limits and all the species tested showed reduced or lack of mortality at 20°C for 7 days and died within a few days at

Table 1. Selected species and their collection caves with *in-situ* measurements of temperature.

Species	Cave	Coordinates	Temperature (°C)
<i>Euryspeonomus eloseguii</i> (Español, 1948)	Cueva de los Cristinos	42°47'51.2"N, 2°14'50.4"W	9.1
<i>Stygiophyes ribagorzanus</i> (Jeannel, 1911)	Cueva de Tossalclases	42°25'28"N, 0°41'28.8"E	10.5
<i>Troglocharinus hustachei</i> Jeannel, 1911	Cueva Negra de Matasolana	42°2'31.2"N, 0°58'22.7"E	13.2
<i>Troglocharinus impellitieri</i> Español, 1955	Cueva Palomera	42°17'10.5"N, 1°10'33.5"E	11.2

23–25°C. Given the phylogenetic relatedness with the species tested here (Fresneda et al. 2024), we assumed a similar thermal response, which is supported by our results (see Results section). Two humidity conditions (100% and 75% RH) were combined with the four temperatures, resulting in eight combined treatments of temperature and humidity. The 11°C and 100% RH treatment was used as a standard control for all species (non-stressful conditions). Although the control temperature does not match exactly the habitat temperature of all the species (see Table 1), we made sure that such small temperature difference did not cause physiological stress to the species. We kept a minimum of 10 specimens of each species under control conditions after the experiment and they remained alive for months (up to 12 months in the case of *T. impellitieri*) showing normal activity and even mating behaviour.

We used programmable climatic chambers with temperature and humidity control (Memmert M360, Germany and Panasonic MLR-352H-PE, Japan). The 100% RH experimental units were covered with a plastic film to prevent desiccation and small holes to provide oxygenation. The 75% RH experimental units were covered with a plastic mosquito mesh, to prevent the beetles from escaping, while maintaining the RH at the 75% value set in the chambers. The humidity in all treatments was monitored in real time (5-minute intervals, for the entire duration of the experiment) with the same Bluetooth equipped Data-Loggers (HOBO MX2301), and the resulting data was exported into .xlsx sheets to check that the conditions did not vary significantly. In every unit, a cotton disc was placed in the centre, to provide a hiding place for the beetles, simulating small crevices or the humid areas beneath rocks that these species encounter in the caves.

The duration of the experiments was 7 days, as in previous thermal tolerance studies with subterranean species of the same lineage and same genera (e.g. Colado et al. 2022a, b). Beetles were fed daily throughout the experiment with humidified fish food pellets. Survival was checked every 24 hours.

Data analyses

Kaplan-Meier survival curves were fitted to visualise and estimate survival probabilities of each species in the different temperature and relative humidity treatments along the duration of the experiment. The average survival time was calculated for each species and treatment, using the restricted mean survival time (RMST) up to a specified time

(tau = 7). This RMST represents the area under the Kaplan-Meier curve within that time window. This calculation accounts for the presence of right-censored data (i.e. individuals that survived on day 7). The survival rate of each species in each treatment was also obtained, expressed as a proportion of survival at the end of the experiment. We fitted a Cox proportional - hazards model (Therneau and Grambsch 2000) to assess the effects of temperature, relative humidity and their interaction on the probability of mortality, using the R packages “survival” version 3.8.3 (Therneau et al. 2024) and “survminer” version 0.5.0 (Kassambara et al. 2024). The Cox proportional - hazards model is a semiparametric model used in survival analysis to examine the effect of covariates (predictor variables) on the hazard rate: the rate at which events happen over time (in this case, death). The hazard function $h(t)$ is the instantaneous rate of the event occurring at time t , given survival up to time t . The higher the hazard rate, the greater the impact of the covariates involved in the events. For each predictor, the model returns a regression coefficient (coef), its standard error (se), a Wald test statistic (z), and an associated p-value. The exponentiated coefficient ($\exp(\text{coef})$) represents the hazard ratio: values greater than 1 indicate increased risk of mortality relative to the reference level, while values below 1 indicate reduced risk (Therneau and Grambsch 2000). For example, a positive coefficient for temperature means that higher temperatures increase the hazard of death, whereas a negative coefficient for the temperature $\times$ humidity interaction indicates that the effect of temperature differs depending on humidity level. The humidity coefficient compares survival at 75% relative humidity against 100%, with large positive values reflecting substantially higher mortality risk at 75%. Significance was assessed using Wald tests for individual coefficients, and global model fit was evaluated with likelihood ratio, Wald, and log-rank score tests.

To test whether a reduction in humidity alone influenced survival at 11 °C, we compared survival curves between 100% and 75% RH within each species. Survival data were analysed using Kaplan–Meier estimates and compared with the log-rank test, which evaluates whether the overall survival distributions differ between groups. In addition, Cox proportional hazards models with humidity as the sole predictor were fitted to estimate hazard ratios, although in cases of complete or near-complete separation (i.e., when survival in one humidity level was uniformly higher or lower), these models produced unstable estimates. Therefore, interpretation was based primarily on the log-rank tests, which are robust to these situations and directly test the null hypothesis of equal survival across treatments.

Finally, we estimated upper thermal tolerance as the mean lethal temperature (LT_{50}), i.e., the temperature at which 50% of the individuals died after the 7 days of experiment, allowing comparison with values reported for other Leptodirini species in previous studies (Colado et al. 2022a). To obtain the LT_{50} , survival data were fitted to both binomial and quasi-binomial generalized linear models, using the package “brglm” version 0.7.2 (Kosmidis 2021), with the same methodology as in Colado et al. (2022a). The final selected model to obtain the LT_{50} value was a binomial model with bias reduction. Due to the lack of survivors in the 75% RH treatments at the end of the experiments (see Results section), the LT_{50} could only be obtained for the 100% RH treatment. All statistical analyses were conducted using R software, v. 4.4.2 (R Core Team 2024).

Data availability

All the datasets and Rscripts used in the analyses are available at Figshare (DOI: 10.6084/m9.figshare.28902560, link: <https://figshare.com/s/48a6b6a148a78c444a0c>).

Results

In all four species studied, survival decreased with increasing temperature and decreasing humidity (Fig. 1). Under control conditions (11°C and 100% RH), most individuals of all species survived the entire duration of the experiment. Although there were a few deaths in the control treatments (two specimens of *S. ribagorzanus* and two specimens of *T. impellitieri*; these are shown in Table 3), these deaths were negligible and probably due to unrelated factors. However, at elevated temperatures (23 °C and 25 °C), no individuals survived 7 days, regardless of humidity.

Across all species and temperatures, a reduction of humidity to 75% caused the death of all individuals in a timespan ranging from 24 hours to 6 days (Table 2). In the 100% relative humidity treatments, the average survival time across all the species and temperatures was 3.58 days. In contrast, in the 75% humidity treatments, the average survival time was only 2.02 days. Thus, a reduction of 25% in the relative humidity caused a reduction of 43% in the survival time on average.

At 11 °C, a reduction of relative humidity to 75% had a dramatic effect on survival in all four beetle species. The log-rank test (Table 5) compares the Kaplan–Meier survival curves between humidity treatments; results are reported as the statistic χ^2 (*log-rank* χ^2) and its associated *p*-value (*log-rank* *p*). The Cox proportional hazards model estimates the relative risk of mortality at 75% RH compared to 100% RH. For this model, *cox coef* is the regression coefficient (log hazard ratio), *exp(coef)* is the corresponding hazard ratio, *SE* is the standard error of the coefficient, *z* is the Wald test statistic, and *cox p* is the associated *p*-value. Very large coefficients and hazard ratios close to zero (e.g. *exp(coef)* $\approx 10^{-10}$) reflect numerical instability due to near-complete mortality in one treatment group. Therefore, inference relies primarily on the log-rank test results, which robustly demonstrate significant differences in survival between 100% and 75% RH for all species (*S. ribagorzanus*: $\chi^2 = 10.25$, *p* = 0.0014; *T. hustachei*: $\chi^2 = 22.81$, *p* < 0.001; *T. impellitieri*: $\chi^2 = 21.0$, *p* < 0.001; *E. eloseguii*: $\chi^2 = 26.07$, *p* < 0.001). In all cases, individuals maintained at 100% RH exhibited very high survival through day 7 (80–100%), whereas survival at 75% RH was severely reduced, with complete mortality in most species.

The Cox proportional-hazards models show that the hazard of death at 75% RH was dramatically higher than at 100% RH for all species (Table 6). The hazard ratios for humidity are extraordinarily large, demonstrating that decreased humidity had an overwhelming negative effect on survival. For instance, in *T. hustachei*, the hazard ratio was 3.47 million, indicating an extreme increase in mortality risk at 75% RH compared to 100% RH. The temperature-humidity interaction was statistically significant

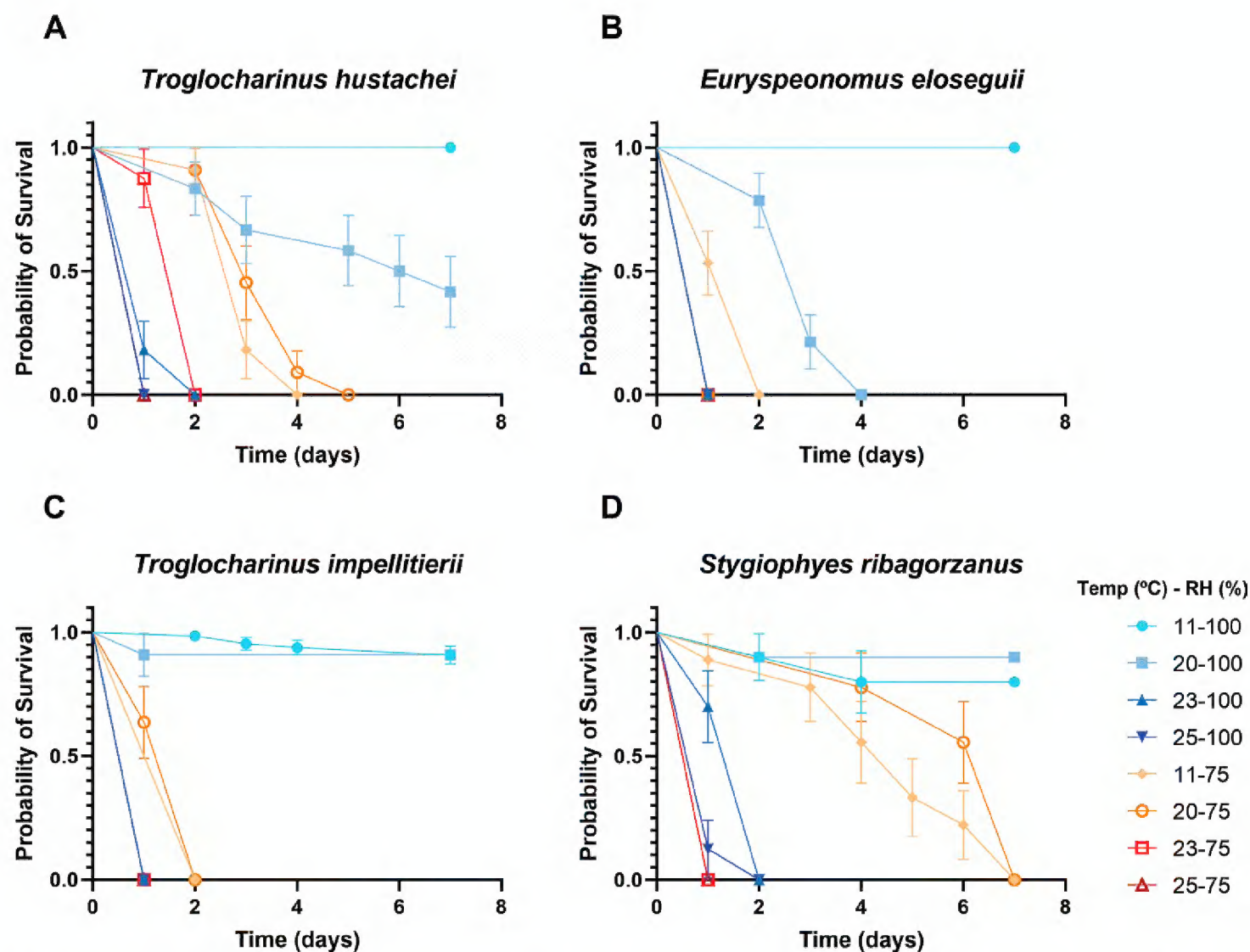


Figure 1. Kaplan-Meier survival curves on the probability of survival of the study species in each combined treatment of temperature (T °C)) and relative humidity (RH (%)).

Table 2. Survival time of each species for the 7 days of exposure at each combined treatment of Temperature (Temp) (11, 20, 23 and 25 °C) and Relative Humidity (RH), (100% and 75%).

Species	RH (%)	Temp (°C)				Average survival time (days)
		11	20	23	25	
<i>E. eloseguii</i>	100	7.00	3.00	1.00	1.00	3.00
	75	1.53	1.00	1.00	1.00	1.13
<i>S. ribagorzanus</i>	100	6.20	6.50	1.70	1.12	3.88
	75	4.67	6.11	1.00	1.00	3.19
<i>T. hustachei</i>	100	7.00	5.25	1.18	1.00	3.61
	75	3.09	3.45	1.88	1.00	2.36
<i>T. impellitieri</i>	100	6.75	6.45	1.00	1.00	3.80
	75	2.00	1.64	1.00	1.00	1.41

in all species. The hazard ratio values < 1 for the interaction coefficients indicate that the detrimental effect of temperature was less pronounced under low humidity conditions (Table 3). This is because the hazard of death was already so high at 75% RH that temperature increases had a comparatively smaller effect on mortality.

The study species showed high sensitivity to increasing temperature and decreasing humidity, but with some differences. *E. eloseguii* exhibited the highest sensitivity

Table 3. Survival rates at 7 days and number of specimens (N) of each species and treatment at the start and at the end of the experiments.

Species	RH (%)	Temp (°C)	N (Day 1)	N (Day 7)	Survival Rate
<i>E. eloseguii</i>	100	11	13	13	1
		20	14	0	0
		23	4	0	0
		25	6	0	0
	75	11	15	0	0
		20	14	0	0
		23	12	0	0
		25	12	0	0
<i>S. ribagorzanus</i>	100	11	10	8	0.8
		20	10	9	0.9
		23	10	0	0
		25	8	0	0
	75	11	9	0	0
		20	9	0	0
		23	5	0	0
		25	8	0	0
<i>T. hustachei</i>	100	11	11	11	1
		20	12	5	0.42
		23	11	0	0
		25	11	0	0
	75	11	11	0	0
		20	11	0	0
		23	8	0	0
		25	10	0	0
<i>T. impellitieri</i>	100	11	12	11	0.9
		20	11	10	0.91
		23	10	0	0
		25	12	0	0
	75	11	12	0	0
		20	11	0	0
		23	10	0	0
		25	12	0	0

to temperature at 100% RH. This species was collected in the coldest cave, at 9.1°C (Table 1), which could be related to its heightened sensitivity. All the individuals of this species survived at 11°C, while all individuals exposed to temperatures of 20°C and above died in less than 4 days (Fig. 1, Tables 2, 3). The Cox proportional-hazards model showed that for every degree of temperature increase, the hazard ratio doubled ($\exp(\text{coef}) = 2.002$), indicating a pronounced thermal sensitivity of these species (Table 6). *T. hustachei*, *T. impellitieri* and *S. ribagorzanus* also demonstrated substantial sensitivity to temperature, but their hazard ratio values were slightly lower, suggesting a somewhat lesser, though still significant, impact of temperature under high humidity (Table 6). These species were collected in slightly warmer caves, as it

can be seen in Table 1. *E. eloseguii* also showed the highest sensitivity to humidity decrease, with no individuals surviving beyond two days at 75% RH. *T. hustachei* and *T. impellitieri* also exhibited 100% mortality under reduced humidity by the end of the experiment, but the mortality response was slower compared to *E. eloseguii* (Fig. 1). *S. ribagorzanus* showed the slowest mortality response at 75% RH, but notably, as with the other species, no individuals survived at the end of the experiment.

Regarding the LT_{50} values, *E. eloseguii* had the lowest (15.4 ± 1.39 °C), while the remaining three species showed a higher and similar LT_{50} , around 19°C (18.9 ± 1.51 °C for *S. ribagorzanus*, 19.6 ± 0.75 °C for *T. hustachei* and 19.2 ± 1.30 °C for *T. impellitieri*). The results and the rest of the output of the LT_{50} model are shown in Table 6.

Table 4. Results of the statistical analyses performed to test for intra-specific differences in the survival patterns within the 11°C treatments, which show only the impact of a decrease in humidity.

Species	N_total	Log-rank χ^2	Log-rank_p	cox_coef	exp(coef)	SE	cox_z	cox_p
<i>E. eloseguii</i>	28	26.07	3.29×10^{-7}	-22.44	1.80×10^{-10}	1.13×10^4	-1.98×10^{-3}	0.998
<i>S. ribagorzanus</i>	19	10.25	1.37×10^{-3}	-2.25	1.05×10^{-1}	8.11×10^{-1}	-2.77	5.57×10^{-3}
<i>T. hustachei</i>	22	22.81	1.78×10^{-6}	-22.57	1.59×10^{-10}	1.38×10^4	-1.64×10^{-3}	0.999
<i>T. impellitieri</i>	24	21	4.59×10^{-6}	-22.31	2.05×10^{-10}	1.25×10^4	-1.78×10^{-3}	0.999

Table 5. Results of the Cox proportional - hazard models for all treatments within each species. The exp(coef) is the hazard ratio.

Species	Predictor	coef	exp(coef)	se(coef)	z	P
<i>E. eloseguii</i>	Temperature	0.693	2.006	0.108	6.455	1.08×10^{-10}
	Humidity	14.56	2.096×10^6	2.471	5.890	3.86×10^{-9}
	Temperature × Humidity	-0.585	0.557	0.104	-5.598	2.17×10^{-8}
<i>S. ribagorzanus</i>	Temperature	0.477	1.611	0.111	4.302	1.69×10^{-5}
	Humidity	8.696	5.979×10^3	2.568	3.387	7.08×10^{-4}
	Temperature × Humidity	-0.363	0.695	0.116	-3.133	1.73×10^{-3}
<i>T. hustachei</i>	Temperature	0.790	2.204	0.112	7.029	2.08×10^{-12}
	Humidity	15.06	3.473×10^6	2.584	5.827	5.64×10^{-9}
	Temperature × Humidity	-0.669	0.512	0.115	-5.837	5.30×10^{-9}
<i>T. impellitieri</i>	Temperature	0.552	1.737	0.104	5.307	1.11×10^{-7}
	Humidity	10.40	3.300×10^4	2.396	4.343	1.41×10^{-5}
	Temperature × Humidity	-0.426	0.653	0.104	-4.107	4.01×10^{-5}

Table 6. Results of the LT_{50} temperature model (binomial model with bias-reduced likelihood).

Species	LT_{50} (°C)	SE	slope	SE	z	P	res_dev	res_df
<i>E. eloseguii</i>	15.4	1.39	-0.743	0.217	-3.419	0.001	1.981	49
<i>S. ribagorzanus</i>	18.93	1.51	-0.282	0.095	-2.981	0.003	35.644	36
<i>T. hustachei</i>	19.58	0.75	-0.886	0.407	-2.177	0.029	17.533	43
<i>T. impellitieri</i>	19.23	1.3	-0.321	0.097	-3.316	0.001	38.574	43

Discussion

Beyond adding evidence to the pattern that subterranean insects are highly sensitive to temperature increase compared to most surface-dwelling arthropods, (e.g., Colado et al. 2022), our study demonstrates that a relatively small reduction of ambient relative humidity, from 100% to 75%, has a severe impact on cave beetle survival, even higher than that of increasing temperature, within the treatments considered in this study.

Cave beetles show an extreme sensitivity to humidity decrease

Our findings show that subterranean species are also highly vulnerable to desiccation stress, an overlooked factor in physiological studies of subterranean species. In the absence of heat stress, all the studied species showed a significant and rapid survival decrease under reduced humidity (75% RH) compared to 100% RH. Insects exhibit a wide range of desiccation resistance mechanisms and strategies (Chown et al. 2011), which are shaped by the environmental conditions of their habitats. Species living in arid environments typically evolve features such as thickened cuticles, increased production of waterproofing cuticular hydrocarbons, reduced respiratory water loss, or behavioural adaptations like burrowing or nocturnal activity to minimize exposure to dry conditions (e.g., Hadley 1978; Gefen et al. 2015). In contrast, insects adapted to wetter habitats often show greatly reduced investment in these protective traits (Menzel et al. 2017). Our results suggest that this is the case for subterranean arthropods. This hypothesis is supported by the few available cuticular studies on subterranean taxa. For instance, the cave spider *Lycosa howarthi* exhibited a lower density of cuticular lipids and a reduced abundance of hydrocarbon classes typically associated with waterproofing compared to its epigeal relatives (Hadley et al. 1981).

Combined effects of desiccation and thermal stress

The responses of the study species to temperature increase under non-stressful humidity conditions (100% RH) were consistent with previous observed thermal tolerance patterns in other species of the Pyrenean Leptodirini clade (Rizzo et al. 2015; Sánchez-Fernández et al. 2016, 2018; Pallarés et al. 2020; Colado et al. 2022a), which are considered among the most heat-sensitive terrestrial arthropods known. Indeed, LT_{50} values for all species were below 20°C, and none of the species survived at temperatures > 20°C (23 or 25°C) for the 7 days of exposure, regardless of humidity levels. Notably, individuals exposed to reduced humidity died in a timespan ranging from 24 hours to 6 days, even at the control (cave) temperature (see Tables 3–5). The deaths in 11°C and 75% RH can only be attributed to the decrease in relative humidity, as the temperature remains at a non-stressful level. This means that their physiological limits are greatly determined by the relative humidity. These results are consistent with a previous study that showed that the upper thermal limits of click

beetles reduce with decreasing humidity (Riddell et al. 2023). The authors suggested this is likely due to a physiological trade-off between limiting spiracular water loss in dry environments and maintaining adequate oxygen intake, ultimately reducing thermal tolerance. Further research measuring cuticular and respiratory water loss in subterranean species would be valuable to elucidate the physiological mechanisms behind the humidity-dependent reduction in thermal tolerance observed here.

Regardless of the underlying mechanisms, our findings have important conservation implications. Reduced heat tolerance, coupled with a limited thermal plasticity (Pallarés et al. 2021), has raised concerns about the high vulnerability of subterranean invertebrates to climate change. Our results suggest that such vulnerability may, in fact, be underestimated, as increasing aridification could further compromise thermal limits and threaten the persistence of these highly specialized species.

Differences in the sensitivity to heat and desiccation stress across cave beetle species

The different sensitivities to environmental changes observed in our experiment among the study species could be related to differences in their evolutionary history and subtle differences in their degree of subterranean specialization, reflected in their morphology. All these four species are specialists of the deep subterranean habitat, and they share the typical troglomorphic traits of the cave-dwelling Leptodirini species (Fresneda et al. 2024), i.e. anophthalmia, absence of wings, reduced pigmentation, elongation of appendages and a reduction of the larval stages (contract or semi-contract life cycle – Cieslak et al. 2014).

Euryspeonomus eloseguii was the most sensitive species, exhibiting the shortest survival times under both humidity treatments and the lowest LT_{50} value, indicating low tolerance to both desiccation and heat. These results align with those previously obtained for its sister species, *E. breuili* (Colado et al. 2022), which shares a similar highly specialized habitat and distribution. *T. hustachei* and *T. impellitieri* showed intermediate sensitivity, with nearly identical LT_{50} values, likely due to their closer phylogenetic relatedness. The least sensitive species to reduced humidity was *S. ribagorzanus*, which had the longest average survival time, although its LT_{50} value did not very similar to those of the *Troglocharinus* species. While *S. ribagorzanus* appears to be the most resistant to both increased temperature and decreased humidity, it is important to highlight that the observed differences are subtle, and this species is still highly sensitive to both temperature increase and reduced humidity.

Our results suggest that even among deep subterranean specialists, small differences in subterranean specialization could be linked to differing physiological tolerances. Future research comparing physiological and morphological data — particularly including species with lower degrees of subterranean specialization (such as those from shallow subterranean habitats, which typically exhibit reduced body size and shorter appendages) — would shed light into the evolutionary relationship between troglomorphy and environmental stress tolerance.

Ecological impact of droughts in cave communities

It could be argued that the humidity conditions tested in our experiments are not realistic in subterranean environments. However, low relative humidity values have been already recorded in some caves in Spain, particularly in warmer and dryer regions, with clear (and already observed) negative impacts on subterranean communities. For instance, in our field observations in the cave of the Sant Gervasi castle, located in the Montsec mountain range (Lleida, pre-Pyrenees region), in July 2023, we recorded 14°C and a relative humidity of 82%, along with dry, dusty soil. We also observed a very low abundance of *Speonomites aurouxi* (Español, 1965), an endemic Leptodirini species which is often abundant in this small cavity (Fresneda and Salgado 2016). Another recent example of droughts affecting the ecological community of caves was documented by Sendra-Mocholí et al. (2025) in southeastern Iberian caves, some of them with historically rich subterranean faunas. In recent visits to these caves, a sharp decline of invertebrate species richness and abundance was observed, with only a few individuals found restricted to the scarce more humid microhabitats left in the otherwise dry (82–86% RH) and dusty caves. Notably, the historically abundant endemic leioid beetle *Anillochlamys tropica* Jeannel, 1909 had almost disappeared in one of the caves. This situation is most certainly a consequence of the severe drought affecting the southeastern Spain, one of the longest and most intense ever recorded in the region. Similar patterns have also been described recently in other Mediterranean regions. A recent study by Galbiati et al. (2025) reported a drastic reduction in abundance and a complete absence of reproduction in populations of the cave spider *Meta bourneti* in north-western Italy following the severe 2021–2022 drought. This further reinforces the view that prolonged droughts, through their effects on subterranean humidity, can severely compromise both population persistence and ecological function in cave systems.

These field observations and studies support the findings of our study, where moderate reductions in relative humidity caused extreme mortality in cave beetles. Notably, abiotic stressors such as elevated temperatures and reduced humidity not only cause immediate mortality but also compromise the ecological fitness of organisms. Longer-term effects could manifest in altered behavioural patterns, suppressed reproductive and foraging activities or an increased susceptibility to pathogenic infections, and could occur at humidity values higher than those tested here. Therefore, further research, focused on the responses at sublethal values of RH (i.e., higher than 75%), would be extremely valuable to better define tolerance thresholds and understand the broader ecological consequences of humidity decline in subterranean species.

Limitations of this work

This study was intended to be a first exploration of the previously unknown topic of the impact of a reduction of humidity in the survival of the cave beetles of the Leptodirini tribe, which has a major relevance in the current context of global climate change and increasing droughts. As such, several limitations should be acknowledged, along with possible directions for future research. First, to ensure comparability with previous studies

on heat tolerance in Leptodirini species (Colado et al. 2022a, 2022b; Rizzo et al. 2015; Sánchez-Fernández et al. 2016; Pallarés et al. 2019, 2021), we used the same temperature treatments, combined with two humidity levels. Due to other logistical reasons (limited number of specimens and experimental climatic chambers – see below) we were unable to test additional temperature or humidity conditions. Related to this, the selection of the same control temperature (11°C) for all species could be questioned, as they were collected from caves with temperatures ranging from 9 to 13 °C (see Table 1). Theoretically, this could induce physiological stress in the species living at higher or lower temperatures than those of the control treatment. Nonetheless, we kept a stock of 10 to 20 individuals of all the four species at 11°C and 100% RH after the experiments, and almost all the specimens remained alive for 6 months in the case of *E. breuili* and *T. hustachei*, and 12 months in *T. impellitieri*. During this time, we even observed mating behaviour in all species, and they fed normally. Therefore, these conditions were apparently not stressful for the selected species, even in the long term. Future studies, however, should explore potential sublethal stress responses (e.g., oxidative stress; see Pallarés et al. 2020) that may arise from relatively small thermal changes. Similarly, testing responses at humidities between 75% and 100% RH would allow to define more precise humidity physiological thresholds for subterranean beetles. While 75% RH was a highly stressful humidity for the tested species in this study, we have also performed additional experiments with cave species at 80% RH (unpublished data), and the specimens show relatively high survival rates under these conditions. This indicates the existence of a narrow critical humidity threshold below which these species cannot persist. Finally, sample size is another important limitation, due to the difficulty to collect large number of specimens of these species from cave populations. However, the sample size in our experiments (N = 10–12 specimens per treatment) was sufficient for performing robust statistical analyses.

Conclusions

Our study reveals that a drop in relative humidity to 75% poses a significant threat to the survival of the four studied Pyrenean Leptodirini cave beetles, even if temperature remained stable at current levels. Considering the direct and rapid mortality observed in our study, exposure to sub-lethal humidity levels above 75% RH most likely cause negative fitness effects on the studied species. Thus, the expected intensification of droughts events could severely impact these already vulnerable species, with restricted distribution and small population sizes, and indeed, field observations have already documented such impact on cave communities. Our findings underscore the need for further research on desiccation resistance in subterranean fauna and have profound implications for the conservation subterranean biodiversity. The narrow thermal tolerances of subterranean species could be further reduced under low humidity. Given that global climate change is not only increasing ambient temperatures but also leading to more frequent and intense droughts, these interactions are especially concerning and should be considered in future vulnerability assessments of subterranean fauna.

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